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Detection of microsporidia in aphid pests associated with cauliflower plants (*Brassica oleracea*)

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Abstract
Microsporidia are eukaryotic obligate endoparasites which are widespread in many vertebrate and invertebrate hosts including insects, mammals, and fish and the infection occurs when a susceptible host ingests microsporidian spores. Microsporidia infect many insects and can also occur in aphid populations, where they may reduce survival, growth, and reproduction. Microsporidia spores are environmentally resistant and can survive outside the host body for about 5-6 years. The spores enters into a susceptible insect host through the gut wall and then spreads to various tissues and organs. In severe infection, they weakened insects leading to mortality of the host. In the present study, the prevalence rate of microsporidian infection in aphids were found to be 30.46 % (78/256). The microsporidian spores were fluorescent in nature, round/oval in shape and showed Brownian movement. The average size (n=5) of microsporidia isolated from aphids were measured as 2.67 ± 0.58 µm × 2.48 ± 0.64 µm (Length × Width). Different developmental stages of the microsporidia also observed under the microscope. The present investigation adds a new host record for microsporidia, thereby extending its known host range.

Keywords: Microsporidia, environmentally resistant, fluorescent, brownian movement, prevalence rate

Introduction

Microsporidia are emerging fungi-like intracellular parasites of economic, veterinary and medical importance which was first recognized almost 169 years ago with the description of *Nosema bombycis* (Naegeli, 1857), a silkworm pathogen. They are having a broad host range infecting both in vertebrates and invertebrates. Till date, about 220 genera and more than 1700 species of microsporidian parasites have been described from various host. It typically affects the apiculture, sericulture and aquaculture industries and in humans, the infections primarily affect HIV-immunocompromised individuals (Stentiford *et al*., 2016; Bojko *et al*., 2022, Kumbhar and Mishra, 2024) [1, 10, 13]. Among invertebrates, they are highly prevalent in arthopods of almost all orders. Further, the prevalence of microsporidia in the insects may vary greatly as per the climatic conditions, and immunity of the host. According to a previous study, the prevalence of microsporidia (M-Dch) isolated from butterfly *Danaus chrysippus* was reported to be highest in rainy season (33.84%), followed by winter (25.92%) and summer (16.92%) season (Kumbhar and Mishra, 2017b) [7]. In insects, infection is initiated when microsporidian spore enter the midgut where their germination is triggered by alkaline intestinal fluids or potassium ions leading to host cell invasion (Nakamura *et al*., 2019) [12]. The infections hinder the fitness of the insects and thus influence the host population size and dynamics. Severe microsporidian infection leads to death of their hosts and in some cases it cause a shift in host sex ratio through feminization of the population, either by male killing, or by turning males into functional females. Since microsporidia is an emerging pathogen with a wide host range, the presence of microsporidia in numerous hosts has not yet been documented. The present study offers the first detection of microsporidia in aphid pests. Additionally, the light microscopy study and prevalence of microsporidia in the aphid pest is also documented in this investigation.

Materials and Methods

Collection of Aphids from Cauliflower Leaves

Aphids were collected from cauliflower leaves grown in the agricultural fields in and around Bhubaneswar, Odisha. They were kept in the insect collection box and stored at 4°C for further screening.

Screening of Aphids for microsporidian infection

The aphids were homogenised in distilled water and filtered through Whatman filter paper. The filtrate was centrifuged at 8000 rpm for 30 min and the pellet was suspended in distilled water and stored at 4° C for further study (Kumbhar et al., 2022) [9]

Prevalence of microsporidia in aphid pests

The prevalence percentage of microsporidian infection in insects was calculated by the following formula:

Light Microscopy observations of fresh microsporidia spores

The infection of microsporidia in aphid pests were determined by examining homogenate smear on a glass slide under trinocular microscope at 400X magnification (Chakrabarty *et al*., 2013) [2]. The size of the spore were determined by using Dewinter Capture Pro software.

Results and Discussion

The cauliflower plants collected for this study were severely infected and damaged by aphid pests. Also different developmental stages of aphid such as nymphs and adults were found throughout the plants (Figure 1). The present investigation report the first detection of microsporidian infection in aphids. The prevalence rate of microsporidia in host varies according to the geographical condition, host density, seasonal variation and transmission mode. In the current study, a total of 65 aphids were screened individually to check the presence of microsporidia, out of which 22 aphid pests were found to be infected with microsporidia. Hence, the prevalence rate of microsporidian infection in aphids were found to be 30.46 % (78/256). Similar findings were reported from lepidopteran insects viz. *Danaus chrysippus* (Plain Tiger Butterfly) and *Samia cynthia ricini* (Eri Silkworm), where the prevalence rate of microsporidia infection was found to be 33.51% and 32.69% respectively (Kumbhar and Mishra, 2022) [9]. In contrast to this, a higher prevalence rate of microsporidia infection (60%) was also recorded in honeybee *Apis mellifera* (Kumbhar and Mishra, 2024) [10] which suggesting that the infection rate is purely host specific and vary according to the ecological conditions. Deviation to the present report, also significantly low percentage of microsporidian infection was recorded in *Danaus genutia* (Striped tiger butterfly), *Melanitis leda leda* as 11.11% and 6 % respectively (Kumbhar and Mishra, 2022) [9]. In another investigation, microsporidia MB was found in *Anopheles* mosquitoes from Busia, Kenya, with an overall incidence of 6.4% (66/1030) (Wandera *et al*., 2025) [14]. Under the microscope, the microsporidian spores were observed as translucent round/oval spores with Brownian movement. The average size (n=5) of microsporidia isolated from aphids were measured as 2.67 ± 0.58 µm × 2.48 ± 0.64 µm (Length × Width). In comparison, the size of isolated microsporidia isolated from aphids are more similar to the standard spore size of *Nosema bombycis* (3.32 ± 0.02 µm × 1.97 ± 0.01µm) strain. Deviation to this, the microsporidia (M-Dch) isolated from the butterfly *Danaus chrysippus* were measured with an average (n=5) size of 3.83 µm ± 0.02 ×2.16 ± 0.01 µm and is ovo-cylindrical in shape (Kumbhar and Mishra, 2017a) [7]. Similarly, microsporidia isolated from larvae of *Spodoptera litura* in Tamil Nadu, India were ovocylindrical in shape and had an average size of 3.91 µm × 1.91 µm (Johny *et al*., 2006) [5]. Mature microsporidia recovered from aphids were oval in shape whereas developmental life cycle stages such as sporoblast, binucleate meront, and tetra nucleate meront stages were observed with distinct characteristics. Some of the black empty spores were also observed indicating that the spore is germinated and spill its sporoplasm to infect adjacent hostcell (Figure 2). Different developmental stages of microsporidia viz. meront (M), sporont (Sp), sporoblast (Spo), mature spore (S) and germinated empty spore (ES) were reported in the gut tissue of honey bee, *Apis mellifera* with distinct identifying characteristics (Kashyap *et al*., 2018, Kumbhar and Mishra, 2024, Grabner and Fiala, 2025) [4, 6, 10]. In addition, binucleate meront, tetranucleate meront and dividing sporoblast stages were also observed Numerous clumps of microsporidian spores were observed which may be associated with their accumulation within host's gut mucus and thus resulting in an unevenly dispersion of spores (Didier *et al*., 1995) [3].

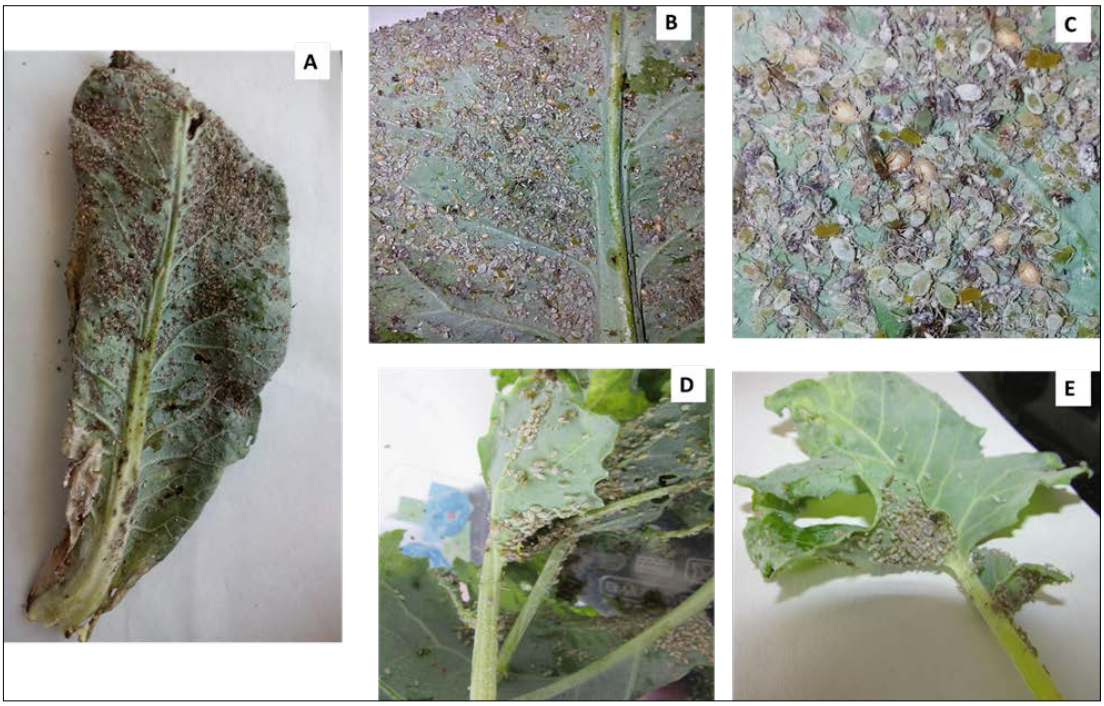

Fig 1: Severity of aphid infestation on cauliflower plants A) Cauliflower leaf entirely covered with aphid pests B) Enlarged view showing dense aphid colonization on the leaf surface C) Aphid population exhibiting different developmental stages including nymphs and adults D) & E) Various parts of cauliflower plants, including leaves and stems, heavily infested and severely damaged by aphid pests.

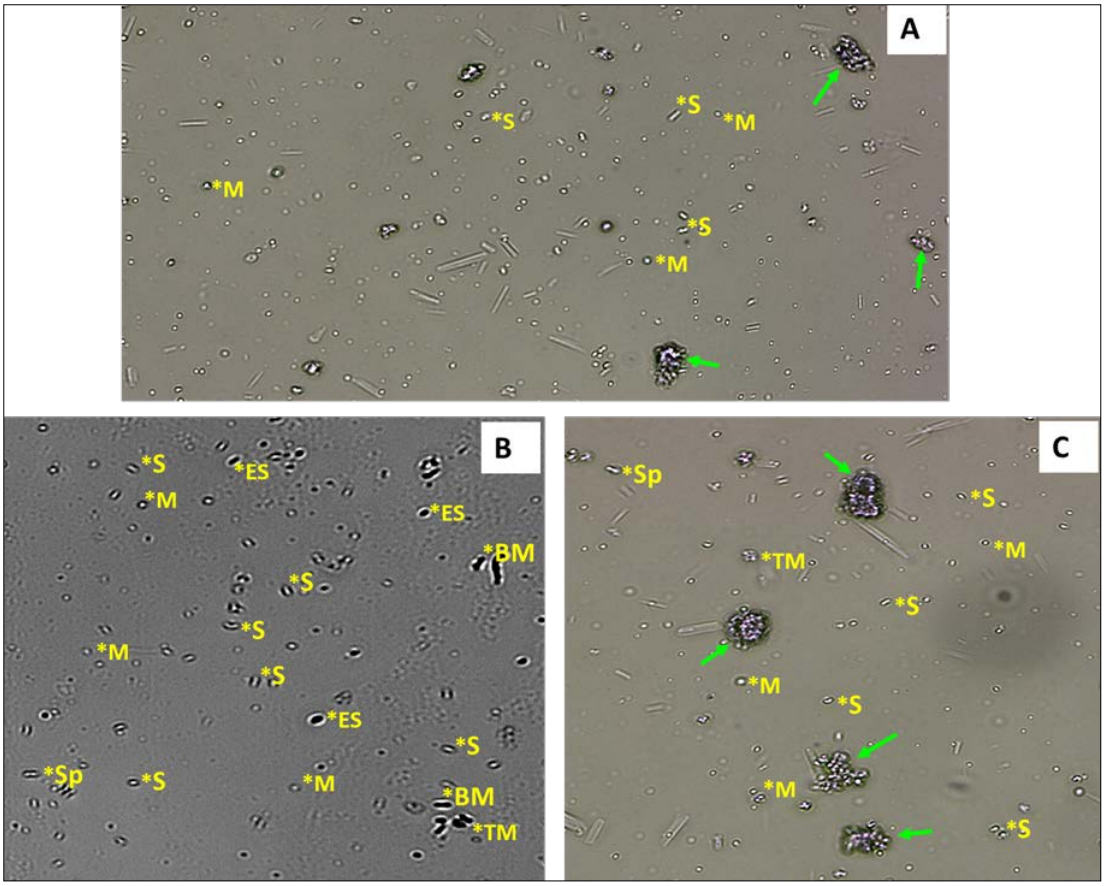

Fig 2: Light Microscopy images (400X Magnification) of microsporidia isolated from aphid pest infesting cauliflower plants A) Homogenized aphid sample showing different developmental stages of microsporidia B) Enlarged view of various life cycle stages of Microsporidian spores: Mature Spore (S), Sporoblast (Sp), Meront (M), Binucleate Meront (BM), Tetranucleate Meront (TM), ES: Germinated Empty Spore C) Green arrow indicating aggregated clusters of microsporidian spores leading to uneven distribution of spores.

Conclusion

The diversity of microsporidia are still largely unexplored, as evidenced by the growing discovery of new microsporidian-host connections across the globe. In the present study, it is concluded that microsporidia are prevalent in aphid populations. Further, this discovery adds to the diversity range of these obligatory intracellular parasites and therefore broadens the host range of microsporidia. Also, research is required to identify the microsporidia species by molecular tools. As microsporidia infections in insects are typically long-lasting and result in modest diseases such as decreased fertility and shorter life spans. Therefore, they are thought to be more beneficial as long-term pest regulators and help to reduce pest outbreaks. This study also boost the idea to develop this microsporidium as a possible biocontrol agent to prevent the infestation of aphid pests in the agricultural field and to determine the influence of environmental factors on its pathogenicity. This finding enhances our understanding of the parasite's host diversity, ecological distribution, and transmission potential, and underscores the need for further studies on its epidemiology and host-parasite relationships.

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